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[Reprinted from BIOLOGICAL BULLETIN, Vol. XXXV., No. 2, August, 1918.]

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THE CYTOLOGY OF THE MYXOMYCETES WITH SPECIAL REFERENCE TO MITOCHONDRIA.[1]

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The Myxomycetes, or Slime moulds, constitute a most interesting group of organisms since they are at once so primitive and so specialized and partake of the distinctive properties of both animals and plants. They stand as a sort of link between the two kingdoms. In the plasmodial phase of their existence, for example, they look like gigantic amœbæ, crawl from place to place, exhibit typical protoplasmic streaming and actively phagocytize foreign particles. In the reproductive phase, on the other hand, they form brilliantly colored fungous-like masses strongly suggestive of plants. It is not surprising, therefore, that they have attracted so much interest among botanists and zoölogists alike. Their general form and nuclear structure has been carefully worked up, but no attempt has been made to extend to them the recent work on mitochondria. This is all the more surprising, because the unique properties of these organisms would lead one to suppose that a careful study of mitochondria in them might yield valuable information bearing upon the Myxomycetes themselves, as well as upon the vexed problem of the general functional significance of mitochondria.

MATERIAL AND METHODS.

The following species of Myxomycetes have been studied:

Arcyria denudata, *Fuligo septica*,
Badhamia ———, *Hemitrichia vesparium*,

[1] Contributions from the Anatomical Laboratory, Peking Union Medical College, No. 2.

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Ceratiomyxa ———,	*Hemitrichia clavata*,
Cribraria ———,	*Lycogala epidendrum*,
Enteridium rozeanum,	*Stemonitis* ———.

They were collected during June, July and August near South Harpswell, Me., and during September, October and November in the vicinity of Baltimore, Md. At South Harpswell, the Director, Dr. Kingsley, very kindly placed the resources of the biological laboratory at my disposal and I wish to thank him for his courtesy.

Portions of the plasmodia were collected, shortly before sporangium formation, on the surfaces of leaves, mosses and damp logs. Immature sporangia were found showing well all the stages between the undifferentiated plasmodium and the young spores. These were placed immediately in the fixative. Smears of the plasmodium were treated in the same manner with results which were only confirmatory, but by no means so distinct or satisfactory.

Fixation:

1. Pieces not larger than 4 cubic millimeters were placed in the following mixture:

Commercial formalin . . . . . 5 c.c.
(A mixture of formaldehyde, water and methyl alcohol should not be used.)
3 per cent. potassium bichromate . . . . . 20 c.c.
4 to 5 days, changing daily.

2. 3 per cent. potassium bichromate, changing every second day, 7 to 8 days.

3. Wash in running water 24 hours.

This is the ordinary Regaud ('10, p. 296) IVB fixative which can be modified with very excellent results, in some cases, by diluting the fixative with an equal volume of water, applying it for 2 to 4 days only and the bichromate for 3 to 5 days as recommended by Sapehin ('15, p. 321).

Another very good fixative is Regaud IVA:

1. Formalin . . . . . 10 to 50 c.c.
Water . . . . . 50 c.c.
for 1 to 5 days.

2. 3 per cent. potassium bichromate, 3 to 4 weeks.

3. Wash in running water, 1 day.

I have found that formalin in 5 per cent. or 10 per cent. solution preserves the normal form of mitochondria better than higher concentrations. The subsequent long mordanting in potassium bichromate may sometimes be omitted with equally satisfactory results.

Other mitochondrial fixatives have been devised containing chromic, picric or osmic acid in varying concentrations. These are the Benda, Champy and Regaud II and III which give very good results, but mitochondria are, I believe, more constant in their response to the Regaud mixtures given above.

Sections should be cut about 3 or 4 μ in thickness.

Staining:

The Heidenhain Iron Hematoxylin Method:

1. 5 per cent. iron alum, 24 hours.
2. Wash in water, 5 minutes or less.
3. 1 per cent. hematoxylin, 24 hours.
   (Made by dissolving 10 gm. hematoxylin in 100 c.c. of absolute alcohol. This should be kept until ripe, when 10 c.c. of the mixture should be added to 90 c.c. of distilled water.)
4. Wash in water, 5 minutes.
5. Differentiate in 2 per cent. iron alum under the microscope.
6. Wash in water at least 1 hour.
7. Pass through 50 per cent., 70 per cent. and 90 per cent. to absolute alcohol, clear in xylol and mount in balsam.

The Bensley Method (E. V. Cowdry, '16b, p. 30):

1. 1 per cent. potassium permanganate, 30 seconds.
2. 5 per cent. oxalic acid, 30 seconds.
3. Rinse in water.
4. Stain in Altmann's anilin fuchsin (anilin water 100 c.c., acid fuchsin 20 gm.), heat once until vapor arises, 6 minutes.
5. Rinse quickly in distilled water.
6. Differentiate in 1 per cent. aqueous solution of methyl green or toluidin blue very quickly.

7. Drain and dehydrate quickly with absolute alcohol.
8. Clear in xylol and mount in balsam.

The Altmann Method:

1. Stain on slide with Altmann's anilin fuchsin, heating once until vapor arises, 6 minutes.
2. Wash quickly in water.
3. Differentiate in alcoholic solution of picric acid (made by mixing one part of a saturated alcoholic solution with two parts of distilled water) until the sections assume a yellowish-pink color.
4. Dehydrate very quickly in absolute alcohol, clear in xylol and mount in balsam.

The Benda Method (*'01, p. 155*):

1. 4 per cent. iron alum, 24 hours.
2. Wash in water, 2 or 3 minutes.
3. Sulphalizarinate of soda, 24 hours. (Made by adding a saturated alcoholic solution of sulphalizarinate of soda drop by drop to distilled water until an amber color is obtained.)
4. Dry excess with blotting paper.
5. Cover with a solution of crystal violet, and warm until production of vapor begins, 5 minutes.

(Made by mixing

1 vol. saturated solution of crystal violet in 70 per cent. alcohol.

1 vol. acid alcohol.

2 vol. anilin water.)

6. Dry off excess with blotting paper.
7. Differentiate with 30 per cent. acetic about 3 minutes.
8. Dehydrate in absolute alcohol quickly.
9. Pass through xylol to balsam.

Observations.

The chief stages in the complicated life history may first be mentioned.

The vegetative phases or plasmodia are found in damp locations, on the surface of logs, fallen leaves and debris. They are

slippery, slimy masses, sometimes nearly a foot in diameter. The color is often very brilliant. For instance, in *Ceratiomyxa* it is white, *Fuligo septica* yellow, *Lycogala epidendrum* light red and *Hemitrichia vesparium* a beautiful dark crimson. But the color is of no generic significance because different species of the same genus exhibit great variability. It is to be noted that they never, under any circumstances, contain chlorophyll, and in this respect they differ sharply from all plants which are not saprophytic and closely approximate to animals. Plasmodia are naked masses of protoplasm containing abundant nuclei, but destitute of cell walls, thus resembling in many ways the syncytia of higher animals and large multinucleate giant cells. They apparently possess all the properties of amœbæ, especially the power of amœboid movement and of being actively phagocytic. They engulf bacteria and foreign particles in much the same way as the so-called macrophages which have been brought into prominence lately in mammals through the use of vital dyes.

In some cases, when the conditions become unfavorable, the nuclei tend to clump together into larger or smaller masses which encapsulate and desiccate. This resting stage, called the sclerotium, may persist for some time. On the resumption of favorable conditions the envelopes are dissolved and the plasmodia reformed.

The reproductive stages are just as remarkable. The plasmodium first migrates to the upper surface of the log or stump or other object, as the case may be, where it will be exposed to more light. It then undergoes great and varied changes in different species. It may form a cushion-like mass, an æthalium, as in *Fuligo;* a flat vermicular aggregation called a plasmodiocarp; or a number of separate sporangia as in *Hemitrichia clavata.* The sporangia may be either sessile or elevated on pedicels. They are surrounded by a definite envelope, termed the sporangium wall, which may even be double. The sporangia contain spores of many hues and varied sculpture and in most cases a capillitium. The capillitium, which is composed of tubes or of threads, generally arranged in the form of a network, is sometimes supportive and may be, at the same time, concerned with the dispersal of the spores. The sporangium wall, the spore wall and the capillitium are all differentiations of the plasmodium.

On germination the protoplasmic content of the spore escapes. It soon becomes actively motile, develops a flagellum and food vacuole and reminds one forcibly of the flagellates. The flagellum is withdrawn and the organism either goes into a brief resting stage (microcyst) or multiplies freely by fission. In the case of multiplication by fission there is a karyokinetic figure and distinctive chromosomes may be seen. Finally these swarm cells clump together and fuse to form another plasmodium.

The granulations about to be described are identified as mitochondria on the basis of the following observations:

1. Their morphology is identical with that of mitochondria in the higher forms of both plants and animals. While they are for the most part spherical, rod-shaped forms do occur.

2. Their distribution is also characteristic. They are generally single but are often arranged in rows like streptococci, or in clumps. They seem to be rather more abundant near the nuclei and about the circumference of the vacuoles.

3. The janus-green reaction is exhibited beautifully by the mitochondria when the contents of the adult spores are crushed out in janus-green solution.

4. They are easily fixed by Regaud's mixtures, as indicated above.

5. They may be stained by the standard mitochondrial methods including the iron-hematoxylin method, the Altmann method, fuchsin and methyl green, and the Benda method.

I have been able to discover no descriptions relating to them in the literature except possibly that of Harper ('00, p. 251, fig. 18), who made a study of cell and nuclear division in *Fuligo varians*, and found certain granules, in a single spore cell only, which he refers to as "granules of reserve material," in a preparation fixed in Flemming's weak fluid and stained with safranin, gentian violet and orange. These granulations present the same morphology as mitochondria, but in the total absence of detailed information, there is no means of ascertaining their nature.

In *Enteridium rozeanum*, we find comparatively large areas of protoplasm in which no differentiations of any sort can be distinguished. There are no nuclei, no mitochondria and but few vacuoles (Fig. 1). Such extensive areas of protoplasm without

visible organization, are, I believe, without counterpart in any other living organisms, outside the Myxomycetes. We must not commit the usual error, however, of calling this protoplasm homogeneous, because homogeneous protoplasm would be incapable of any vital manifestations or of activity of any sort. They stain light gray with iron hematoxylin, green with fuchsin methyl green, yellow with the Altmann method, and a dull pink by the Benda method. Around their margins groups of nuclei with a few mitochondria may be seen and a greater tendency toward vacuolation is noted.

In other parts of the plasmodium the mitochondria are fairly abundant (Fig. 5). They are for the most part spherical and of quite uniform size, varying between 0.25 and 0.5μ in diameter. The absence of the really tiny mitochondria, to be seen occasionally in higher forms, should be noted. Since the mitochondria never exceed these limits, the possibility of plast formation may be definitely excluded, except perhaps along the margins of the capillitial vacuoles. Rod-like mitochondria occur but they are quite rare. Filamentous, net-like and ring forms were never seen. The spherical mitochondria often clump together in pairs like diplococci or in linear series like streptococci. There can be no confusion with bacteria, however, because of their perfectly definite and characteristic reactions to fixatives and stains, which have already been mentioned. They are likewise spherical and look quite homogeneous. They stain quite darkly with iron hematoxylin and undergo definite modifications with the approach of spore formation, to be described subsequently. The ground substance is but faintly vacuolated and the nuclei are scattered irregularly, but fairly evenly.

Sometimes the vacuolation of the ground substance is much more marked, as is illustrated in Fig. 3. The vacuoles themselves are quite large and are usually, though not always, spherical. They often seem to run together. They contain for the most part a colorless liquid, never distinct spherules of protein as in the so-called "vacuoles de ségrégation" of Renaut and Dubreuil. Their walls are merely separation membranes and cannot be distinguished except in *Cribraria* where well formed and quite thick boundaries occur.

The mitochondria, which are also quite numerous, are frequently clumped about the periphery of the vacuoles, but they are never to be found within them, and this is another and very important distinction between mitochondria and bacteria which are phagocytized by Myxomycetes and are segregated and digested inside the vacuoles. The heaping up of mitochondria about the vacuoles may be merely a surface tension phenomenon. Their accumulation about the nuclei represented in Figs. 1, 3 and 4 may also be due in part to surface tension. It is important to bear in mind that the areas of clear protoplasm are, in their deeper parts, devoid of both mitochondria and vacuoles which become more numerous as we approach the nuclei, and that we may be dealing with nothing more than a heaping up of mitochondria in foci of more rapid metabolism.

Still more abundant mitochondria are shown in Figs. 4 and 6 but the vacuoles are fewer. Different parts of the organism are often separated by irregular, dense and homogeneous septæ, not related to capillitium threads, one of which is illustrated. It often happens that stretches of protoplasm separated in this way may exhibit a difference in the intensity of staining, the extent of vacuolation or in the number of mitochondria.

Fig. 2 of *Lycogala epidendrum* shows a portion of the active protoplasm of the plasmodium migrating upward through the interstices of the hypothallus to the æthalium where the spores will be formed. The nuclei are still spherical and of about the same size, but contain more distinct and prominent nucleoli. The mitochondria show little tendency toward perinuclear condensations. The protoplasm appears to be of rather open texture. The hypothallus presents an ill-defined fibrous appearance and is quite devoid of mitochondria and nuclei.

Spore Formation:

The early stages in spore formation in *Badhamia* are illustrated in Fig. 8. The nuclei, which were so conspicuous in the plasmodium, can no longer be seen with the aid of mitochondrial methods of staining. Their loss of affinity for iron hematoxylin and other basic dyes calls to mind the condition of affairs in oögenesis of certain animals where there is a temporary dis-

appearance of basophilic chromatic material. The ground substance of the protoplasm presents a sort of flocculent appearance, being denser in some regions than in others. The first indication that spore formation is taking place is to be seen in the clumping of the mitochondria. All the mitochondria are in the form of spherules of fairly uniform size. They stain with equal intensity with iron hematoxylin. The differences in shade illustrated are indicative of perspective only. Soon the little clumps of mitochondria become surrounded by a membrane, the first spore membrane, which stains only faintly with iron hematoxylin. Traces of nuclei can be seen in some of these early spores. Spore formation proceeds from a definite center so that it is possible to see many stages in a small area. Fig. 8 is taken through such an area, the early stages being above and the later stages below. In certain other Myxomycetes, however, spore formation is said to take place simultaneously throughout the whole sporangium.

Rather more advanced stages in *Fuligo septica* are shown in Figs. 7 and 9. Here all the spores are well formed and discrete and surrounded by membranes. The nuclei have reappeared. They are spherical and stain diffusely. It is difficult to distinguish any nucleoli within them, since they are of about the same size as the mitochondria. There is no apparent change in the mitochondria. The ground substance of the protoplasm presents the same flocculent appearance with a marked tendency toward the production of vacuoles in some of the spores.

Very profound changes now take place. In *Fuligo septica* (Fig. 10) the spores lose every trace of their membranes and degenerate into naked, nucleated masses of protoplasm which are distinctly smaller than in the preceding stage. They also lose their spherical or oval shape and become often quite angular. Their nuclei stain so intensely with iron hematoxylin that it is difficult to make out any structure in them. The ground substance is more homogeneous and stains diffusely and evenly. The appearance of the mitochondria has completely changed. Instead of occurring in the form of large spherules they are now considerably smaller, rod-like and sometimes almost filamentous. They often clump about the nuclei in a manner suggestive of

certain stages in the spermatogenesis of mammals. But they may be quite uniformly distributed through the cytoplasm or else condensed to one side of the nucleus only. Different spores vary greatly in this respect. Sometimes the spores stain so intensely that the mitochondria can only be distinguished with difficulty. The large clear vacuoles and tortuous spaces which occur in some of the spores indicate that it might be worth while to study this material with methods adapted to the demonstration of the vacuolar apparatus. The conditions in *Badhamia* are identical except for the fact that the mitochondria in the spores are much more numerous and filamentous.

Other alterations accompany the formation of the definitive spore capsules which are best seen in sections of *Enteridium rozeanum* (Fig. 11). The capsules are quite complicated in structure. At first sight they seem to be covered with spines but Fig. 11 shows that the spines are in reality the walls of little compartments set upon a homogeneous basement membrane. It often happens that the substance of the spore shrinks away from the membrane. Sometimes this is followed by the partial collapse of the membrane itself. The contents of these mature spores are important. The nuclei seem to be much broken down and traces of them can be distinguished only after careful study. The mitochondria have, almost uniformly, reverted to their original spherical shape and can easily be stained in the usual way with janus green, if the spore contents are squeezed out in a solution of 1 : 10,000 of the dye.

The entire process of spore formation is subject to great variation in different Myxomycetes. In *Arcyria denudata*, for instance, the process is quite different from that which I have described in *Badhamia*, *Fuligo* and *Enteridium*. The first sign of approaching spore formation is a segregation and encapsulation of comparatively large masses with abundant nuclei and many mitochondria. Instead of being hard to distinguish, the nuclei are quite conspicuous with well-defined chromatin networks and nucleoli. Through successive divisions these multinucleated masses become smaller and smaller. As the final division approaches, the walls become thicker and the nuclei stain less intensely, as is shown in Fig. 12. Division is by mitosis. The

structure of the spores is very intricate and is best brought out when stained with fuchsin and methyl green. The nuclei are large and faintly stained and usually present a solitary spherical nucleolus which likewise stains but faintly. There is often, though not always, present in the cytoplasm an irregular mass of material surrounded by a vacuole. The material can be clearly resolved into acidophilic and basophilic constituents, staining with fuchsin and methyl green respectively. Of the two, the basophilic structures are the most definite and appear in the form of definite spherules suggestive in some measure of chromosomes. But the occurrence of the material in vacuoles is equally suggestive of phagocytosis, which, however, would seem to be unlikely in view of the dense membrane surrounding each spore. Only further study will reveal the nature of the material. Mitochondria are distributed quite evenly throughout the remainder of the cytoplasm and are characterized by their rod-like shape. There is no counterpart here for the changes in the morphology of mitochondria observed in *Badhamia*, *Fuligo* and *Enteridium*.

No special provision is made during spore formation, or in cell division generally, for an equal division of mitochondria, which might be looked for if we regard them as in any sense carriers of heredity. They do not, like the nucleus, change their solubilities or staining reactions during spore formation. This is the more interesting since the resistance of the mitochondria increases progressively in the spermatogenesis of mammals. There is some indication, however, that with spore formation there is a tendency toward a reduction in the amount of mitochondria with relation to the cytoplasm.

Capillitium:

I have not traced the formation of the capillitium from confluent vacuoles as described by Strasburger but my observations of the later stages are of interest in connection with the work of Harper and Dodge ('14, p. 3). In plasmodia of *Hemitrichia clavata* and *rubiformis* fixed in weak Flemming and stained with either safranin, gentian violet and orange G, or with iron hematoxylin, they describe lines running toward the vacuolar tube, where their centers of convergence are marked by a series of granulations (see their Figs. 1–5).

In my own material of the same species, I have been unable to find the radiating lines and perhaps this may be due to the fixative which I have used, but I have observed the granules. The granules in my preparations fixed in Regaud's fluid and stained with iron hematoxylin are almost indistinguishable from the mitochondria which I see in the surrounding plasmodium (Fig. 13). They are of the same shape except in some cases where they are in very intimate contact with the vacuolar membrane and flatten out upon it, which may be due to surface tension. It is important to note that the mitochondria are not illustrated in Harper and Dodge's figures which would lead one to suppose that they have been destroyed by the fixative; so that our conclusion is warranted that the granules which surround the vacuole and constitute the termini of the lines are more resistant than the mitochondria to fixation. It is probable that the destruction of the mitochondria may have been occasioned by the acetic acid in the Flemming's fluid. We know that plasts generally are more resistant to acetic acid than are true mitochondria, which would lead us to suppose that the granules in question are plast-like. This interpretation falls well in line with Harper and Dodge's explanation of the significance of the radiating lines. They regard them as "pathways by which materials are brought in from the surrounding cytoplasm." I cannot agree with them, however, in their interpretation of the significance of the nuclei as morphogenic factors. I would be inclined, on the contrary, to regard the granules as plast-like and perhaps truly formative, as in the higher plants.

Furthermore, Harper and Dodge ('14, p. 7) have described certain interesting formations within the lumen of the capillitial tube. They say that:

"The granular material in the interior of the capillitial thread (Figs. 1 and 2) becomes less as the wall thickens and the spirals appear, and as the thread matures it practically disappears (Figs. 3 and 4). There is, of course, no evidence that granular material as such passes from the interior of the thread into the forming spirals. We are inclined to suspect that the stainable granules in the interior of the thread are precipitation products formed in fixation, and that in the living condition the capillitial

cavity contains only materials in solution in the cell-sap. These materials may be used up in the formation of the capillitial wall and spirals so that in late stages no such precipitation products are formed."

In the first place my observations show, contrary to Harper and Dodge, that the granular material is quite abundant in well formed capillitial threads. In Fig. 17 of *Arcyria denudata* the granules are very numerous and occupy almost the whole interior of the thread. In fact I have never found them to be so abundant in early stages in the formation of the capillitial threads in *Hemitrichia clavata*. Neither have I found any indication that granular material as such passes from the interior of the thread into the forming spirals (see Figs. 13, 15, 17, 18 and 19). It is hard for me to believe that these granules are precipitation products formed by fixation. It will be noted that they are often of astonishingly uniform size and shape which one would not expect in products of precipitation or coagulation. Moreover they are often absent or isolated or distributed evenly over the interior of the thread showing little tendency to clump, which one would likewise expect in the case of products of fixation. They do resemble mitochondria very closely. Their rod-like and even filamentous shape is well illustrated in my figures. They are often arranged in rows, suggestive also of mitochondria. They are usually about the same size as mitochondria but they are sometimes larger, as shown especially in Fig. 17. Their staining reactions also are suggestive of mitochondria, for they take the fuchsin as well as the iron hematoxylin, after fixation in Regaud's fluid. Nevertheless we cannot consider them to be mitochondria because they occur within the capillitial tubes, quite apart from nuclei and from protoplasm. It would appear more probable that they constitute merely a part of the material brought in, which is useless in the formation of the capillitial wall.

Harper and Dodge refer to two types of nuclei at this stage in *Hemitrichia clavata* and I have been able to confirm their finding as illustrated in Fig. 19. It will be seen that the majority of the nuclei are large and pale and have definite nucleoli. Some, however, are smaller and much more intensely stained with the hematoxylin: these Harper and Dodge regard as undergoing

degeneration. They are distributed quite evenly throughout the plasmodium which definitely precludes the possibility that they may result from mechanical injury. In preparations stained with fuchsin and methyl green, the small nuclei take the fuchsin to a remarkable degree, behaving just like the nuclei of the spores, which stain in precisely the same way. This points, perhaps, to the conclusion that these small nuclei are undergoing differentiation with a view to spore formation.

Sporangium Wall and Hypothallus:

My observations do not bear upon the question of the mode of formation of the sporangium wall and hypothallus. In all probability both of them are differentiations or secretions of the plasmodium as is generally supposed. I have found no indications that the mitochondria play any part in their formation. They are at first gelatinous and afterwards become membranous. A good account of sporangium formation in *Trichia* and *Arcyria* is given by Kranzlin ('07, p. 179).

With this progressive differentiation of spores, capillitium, sporangium wall and hypothallus, there is a distinct and gradual alteration in the plasmodium itself. At first it usually contains comparatively large quantities of debris, the more solid portion of which is left behind in its path and persists to some extent in the hypothallus. There is a further segregation with the formation of differentiated products, the end result being that the protoplasm of the spores is comparatively free of foreign material.

Discussion.

We are inclined to divide living organisms into two groups, plants and animals, and perhaps unconsciously to assume that this classification is sufficient. It is interesting to find that the Myxomycetes, or slime moulds, cannot be dismissed so easily, for they partake of the properties which we have been inclined to regard as distinctive of plants, on the one hand, and of animals, on the other. Great difference of opinion is manifest in the literature. At first they were considered to be plants and were called *Myxogastres*, in 1829, by Fries, who grouped them among the fungi. The word Myxomycetes also indicates their fungous-

like properties. With the discovery of the plasmodium, opinion changed and the term *Mycetozoa* was introduced. Even now there is absolutely no concensus of opinion on the subject of their relationships. Coulter, Barnes and Cowles ('10, p. 1) place them tentatively among the first of the *Thallophytes*. Chamberlain ('15, p. 152) groups them with the *Schizophytes*. Torrend[1] and Schintz[2] conclude that the *Myxomycetes* are related to *Fungi* rather than to animals. And finally Osborn ('11, p. 339) Schwartz ('14, p. 238), Harper ('0c, p. 235) and others associate them with the Plasmodiophoraceæ, Chytrideæ and Acrasieæ; while Maire and Tison[3] relate them to the Sporozoa, Elliott[4] to animals generally, by reason of their feeding reactions, and Parker and Haswell ('97, p. 61) classify them as intermediate between the Rhizopoda and the Mastigophora.

We may summarize their plant-like features as follows: In the first place they form brilliantly colored fungous-like masses strongly suggestive of true fungi. The capillitial threads which are found in the sporangia frequently play an important part in the dispersal of the spores and remind one of the elaters of liverworts. If Harper and Dodge ('14, p. 9) are correct, however, there would appear to be a closer analogy between the capillitium and the protozoan endoskeleton, since they believe them both to be formed by a process of intraprotoplasmic secretion. They bear also a certain superficial resemblance to the puff-balls, which have likewise a mass of spores supported by a capillitium-like framework and contained in a sporangium. Pinoy ('08, p. 630) records a sexual dimorphism in *Didymium* which reminds him of the condition in Mucor as described by Blakeslee. Moreover the supposed presence of cellulose in the sporangium walls, the spore walls and the cyst walls of the sclerotium indicate a plant affinity. And finally the possession of well-defined spores is cited as evidence that they should be regarded as plants.

Yet they apparently resemble animals just as closely and for this reason they have been called "Mycetozoa" (fungus-animals),

[1] Reviewed in *Jour. Roy. Micr. Soc.*, 1910, p. 221.
[2] *Jour. Roy. Micr. Soc.*, 1914, p. 292.
[3] *Jour. Roy. Micr. Soc.*, 1909, p. 626.
[4] *Jour. Roy. Micr. Soc.*, 1917, p. 500.

a term which indicates a compromise, and is perhaps preferable. We may enumerate briefly their many points of resemblance to members of the animal kingdom. It has already been mentioned that the plasmodium behaves to all intents and purposes like a gigantic amœba. It moves freely from place to place, burrows deeply in rotten wood and into the substrata when it is necessary to obtain nutriment, and, most significant of all, it is capable of phagocytosis. That is to say, it can actively devour and digest bacteria and other foreign bodies which it is able to engulf. In the total absence of chlorophyll, the green pigment so characteristic of plants generally, and the accompanying saprophytic behavior, they resemble the fungi, bacteria and other saprophytic plants, on the one hand; and the whole animal kingdom on the other, with but few exceptions. Following a study of the feeding habits of *Badhamia* Elliott[5] looks on Mycetozoa as parasites, more animal than vegetable. The swarm cells with their flagella and food vacuoles, their motility and power to multiply by fission call to mind the Flagellata.

To these points of similarity to animals may now be added the mitochondria, which I have found to occur in all the species of Myxomycetes which I have examined, numbering ten or more. The mitochondria observed are identical, so far as can be ascertained, with the mitochondria in the higher plants and in the whole animal series from the protozoa to man. The point is, that in some lower plants, the mitochondria are apparently totally absent or else quite different from those which I have described in the Myxomycetes. They have not been described in the Cyanophyceæ; in the bacteria their presence is doubtful (E. V. Cowdry '16a, p. 433), and in the Chlorophyceæ they have been found in but few forms: Guilliermond ('13, p. 86) thinks that here the enlarged chloroplast takes over their function. So that the mitochondria of the Myxomycetes approximate far more closely to the mitochondria of animals than to those of the lowest plants.

The discovery of mitochondria in the Myxomycetes extends our knowledge of the extraordinary breadth of distribution of these granulations in living matter. I have already shown ('17, p. 225) that, so far as our present methods of technique go,

they are identical in animals and in plants. Champy's ('11, p. 154) statement that "I would not regard as living a cytoplasm which does not contain mitochondria" is rather radical in view of our knowledge of the structure of the lowest plants. The bacteria and the Cyanophyceæ are of special interest in this connection. Furthermore, it is common to find comparatively large stretches of protoplasm in certain of the Myxomycetes which contain no mitochondria. While we cannot say how active this protoplasm is we cannot regard it as totally inert and lifeless.

Concerning the continuity of mitochondria in the Myxomycetes it may be said that they invariably occur in the plasmodia as well as in all stages of spore formation, even to the adult spore. While I have not yet studied the swarm spores, it is extremely probable that mitochondria occur in all stages of the life cycle. No indications were observed of *de novo* formation of mitochondria though it is highly improbable that if such occurred they would have been detected. It seems unnecessary to assume, as some workers have done, that we must find mitochondria grading into the invisible in order to demonstrate a *de novo* origin, because it is possible that the mitochondrial aggregate must attain to a certain size before acquiring characteristic density and staining reactions.

The cyclical changes in the morphology of the mitochondria suggest similar changes which have already been described, long since, in higher forms. The most striking of these is the change from the large spherical mitochondria of the youngest spores to the smaller rather rod-like ones of those which are more mature. They resume their granular condition in the mature spores. They are often rod-like and arranged parallel to the direction of the current in streaming protoplasm of active plasmodia. Their clumping about the nucleus and their whole behavior make it very plain that their morphology and distribution are governed by the same laws here which operate in animals and in the higher plants, whatsoever they may be.

It is interesting to note, *a propos* of current statements to the effect that the mitochondria are transformed into cellular differentiations, that, so far as can be ascertained, they play no

active part in the formation of the sporangium wall, the complicated spore capsules and capillitia, the hypothallus, the pigment and lime deposits of the Myxomycetes.

The occurrence of typical mitochondria in the active plasmodia, crawling from place to place, as well as in all stages in spore formation even to the fully mature spore, surrounded as it is with a thick restraining horny capsule must indicate one of two things: either that the mitochondria are active in some of these locations and passive in others or else that they take part in the activities of the cell in all stages. The second supposition is usually granted by workers on mitochondria in higher forms. It is apparent, then, that this study of the Myxomycetes materially supports the general supposition that the mitochondria are concerned in some fundamental vital process common to all living matter, perhaps with protoplasmic respiration and possibly with growth.

Conclusions.

1. Mitochondria occur in *Arcyria*, *Badhamia*, *Ceratiomyxa*, *Cribraria*, *Enteridium*, *Fuligo*, *Hemitrichia*, *Lycogala*, *Stemonitis* and probably in all other Myxomycetes.

2. The mitochondria in Myxomycetes so far as can be ascertained differ in no wise from those occurring in the majority of plants and in all animals.

3. The Myxomycetes resemble the lower animals much more closely than they do the lower plants with respect to their mitochondrial content.

4. The Myxomycetes offer a unique opportunity in many ways for the experimental study of mitochondria.

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Explanation of Plate I.

All the figures have been drawn with Zeiss apochromatic 1.5 mm., compensating ocular 8 and camera lucida. They have been reproduced without reduction so that the magnification as they now appear on the plates is 2,600 diameters.

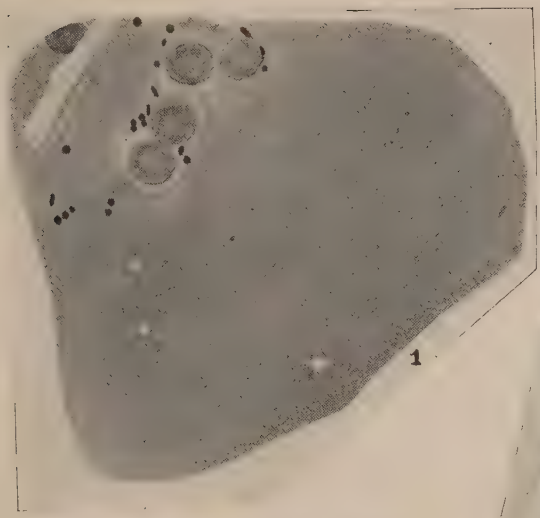
Fig. 1. *Enteridium rozeanum* fixed in Regaud's fluid and stained by the Benda method. The mitochondria are stained a dark purplish-blue color against a pink background. Plasmodium showing a large area of apparently homogeneous protoplasm, a few spherical nuclei and some mitochondria, are seen to one side. The ground substance shows a tendency to be more vacuolated and to stain fainter in the vicinity of the nuclei.

Fig. 2. *Lycogala epidendrum* fixed in Regaud's fluid and stained with iron hematoxylin. The mitochondria are stained bluish-black against a gray back ground. A portion of the plasmodium crawling upward toward the æthalium on the meshwork of the hypothallus.

Fig. 3. *Enteridium rozeanum* same fixation and stain as Fig. 1. Likewise a portion of the plasmodium greatly vacuolated. The nuclei are stained intensely and the mitochondria are often grouped about them as well as about the vacuoles.

Fig. 4. The same showing quite well differentiated fibrous structures which often appear to be of the nature of septæ separating the plasmodium into different areas.

Fig. 5. Another part of the same plasmodium with scattered nuclei and mitochondria of various forms. The ground substance shows an indistinct vacuolation.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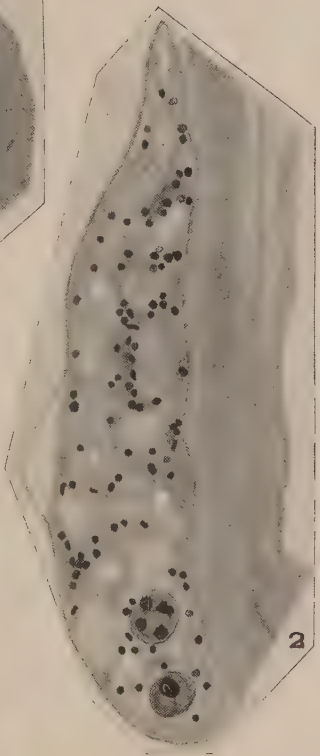
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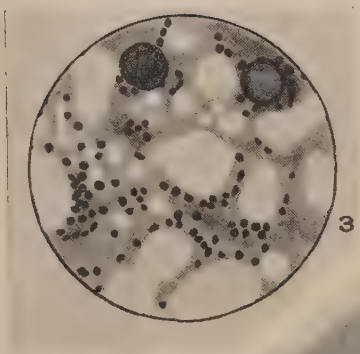
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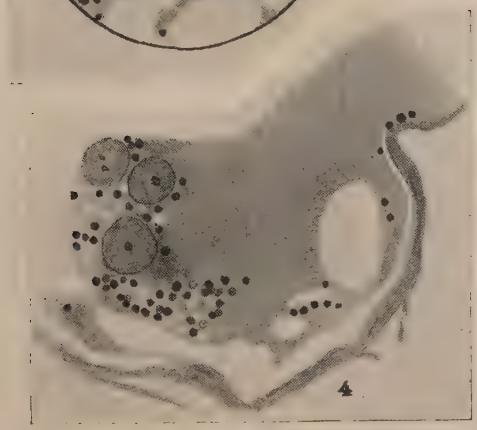
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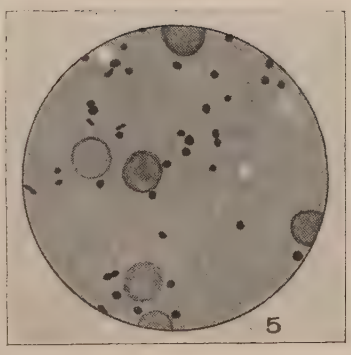
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Explanation of Plate II.

Fig. 6. *Enteridium rozeanum* fixed in Regaud's fluid and stained by the Benda method, mitochondria blue-black and ground substance pink. Another septum is shown at the side. The mitochondria occur in distinct clumps sometimes about the nuclei. The apparent differences in the staining reactions of the mitochondria simply indicate depth in the preparation.

Fig. 7. *Fuligo septica* fixed in Regaud's fluid and stained with iron hematoxylin; mitochondria blue-black and ground substance gray. Immature spore with a faint nucleus and conspicuous mitochondria.

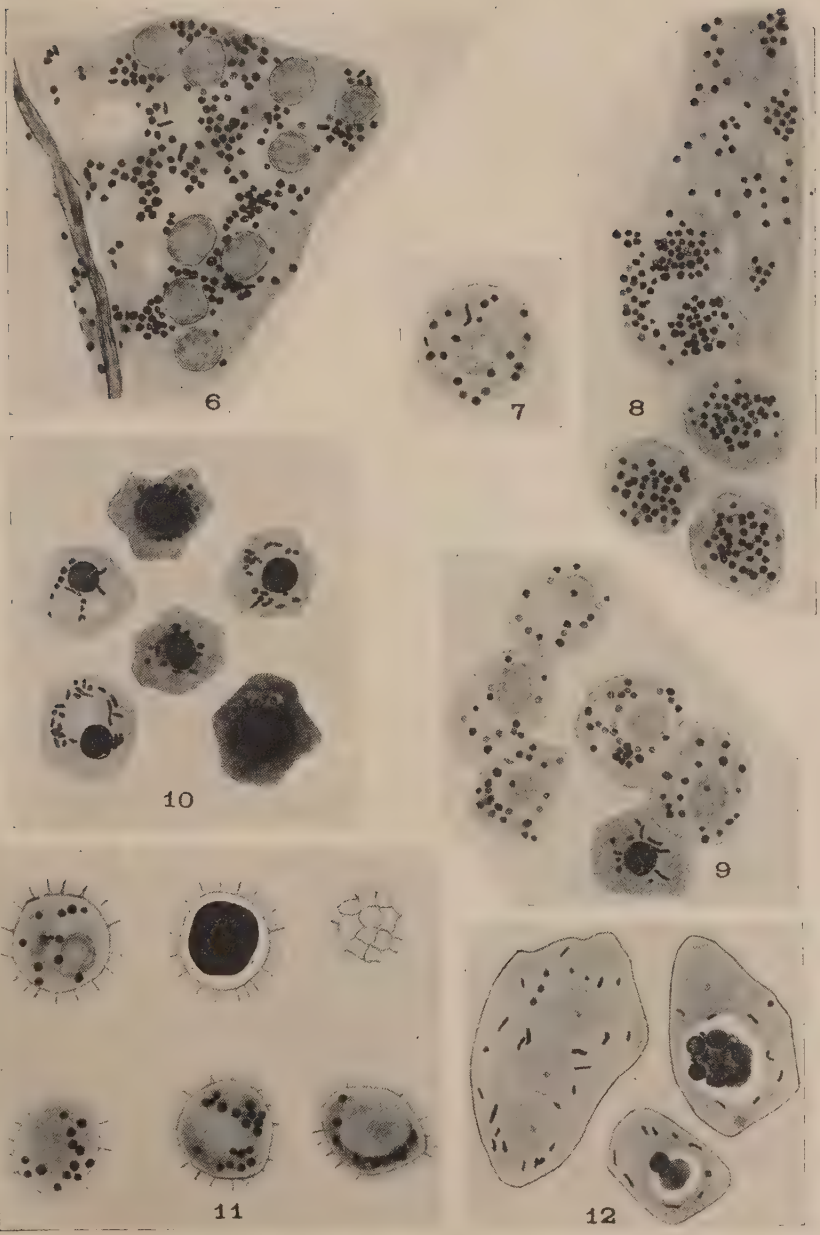
Fig. 8. *Badhamia* ————, fixed and stained in the same way. It shows the first stages in spore formation. The nuclei are indistinct; the mitochondria show progressive clumping and the spore membranes are gradually formed.

Fig. 9. *Fuligo septica*, same fixation and stain. More mature spores of smaller size, without a distinct cell membrane, with strongly stained nuclei and more rod-like mitochondria. The mitochondria often closely approximate to the nucleus. The ground substance is more homogeneous than in the preceding stage and more deeply stained.

Fig. 10. *Fuligo septica* fixed and stained in the same way showing rather later stages in spore formation than Figs. 7 and 8. The spores are surrounded with a definite membrane. They have faintly staining nuclei and spherical mitochondria. One of them is rather more advanced.

Fig. 11. *Enteridium rozeanum* same fixation and stain. Quite advanced spores with definite capsules. The nuclei are faintly stained and the mitochondria spherical and in one case clumped near the nucleus.

Fig. 12. *Arcyria denudata* fixed in Regaud's fluid and stained with fuchsin and methyl green. The mitochondria are crimson against a greenish background. One cell not finally divided containing three nuclei and two others each containing a mass of material in a vacuole. The material consists of a basophilic part, staining green, definitely delimited in the form of spherules, and of a more irregular acidophilic mass staining red.



N. H. COWDRY.

Explanation of Plate III.

Fig. 13. *Hemitrichia clavata* fixed in Regaud's fluid and stained with iron hematoxylin. The mitochondria are blue-black and can with difficulty be distinguished from the granulated ground substance. Plasmodium, within the sporangium, showing an early stage in the formation of the capillitium. The spirals are beginning to appear.

Fig. 14. *Arcyria denudata* fixed and stained in the same way. These cells are in the stalk, next the envelope. They will apparently take no part in spore formation. They show indications of cytolysis and contain but few mitochondria.

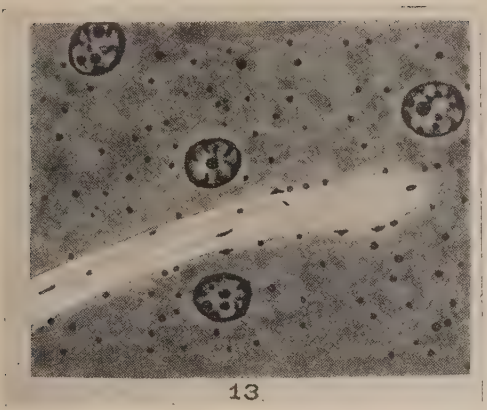
Fig. 15. *Hemitrichia vesparium* fixed and stained in the same way. Spores and cross and longitudinal sections of capillitium.

Fig. 16. *Hemitrichia vesparium*, same fixation and stain. A portion of the plasmodium flowing between the meshwork of the hypothallus. The mitochondria are elongated in the direction of movement. There is also a faint striation in the ground substance in the same direction. Some of the nuclei have well marked nucleoli.

Fig. 17. *Arcyria denudata* fixed in Regaud's fluid and stained with fuchsin and methyl green. The mitochondria are crimson. There are two immature spores which apparently contain a comparatively large amount of golden yellow pigment. The terminal enlargement of the capillitial thread also contains a series of granulations staining just as the mitochondria do.

Fig. 18. *Hemitrichia clavata* fixed in Regaud's fluid and stained with iron hematoxylin showing also internal granulation.

Fig. 19. *Hemitrichia clavata* fixed and stained in the same way. Plasmodium with cross section of capillitial thread and two types of nuclei, the smaller ones staining darkly and the larger ones lightly.



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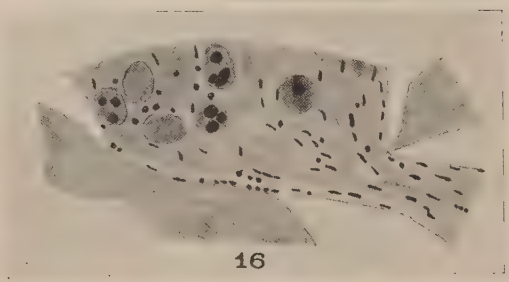

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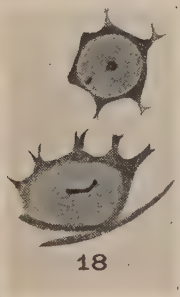

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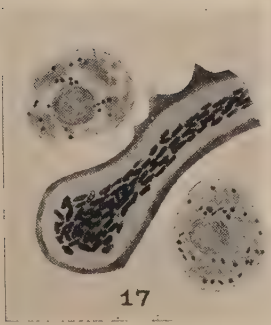

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